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L3: Entry 1 of 1

File: USPT

Apr 11, 1995

US-PAT-NO: 5405941

DOCUMENT-IDENTIFIER: US 5405941 A

**** See image for Certificate of Correction ****

TITLE: MEKK protein, capable of phosphorylating MEK

DATE-ISSUED: April 11, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Johnson; Gary L.	Boulder	CO		

US-CL-CURRENT: 530/350; 435/69.1, 536/23.1

CLAIMS:

What is claimed is:

1. A substantially pure protein comprising the amino-acid sequence, as set forth in SEQ ID No. 2.
2. The protein of claim 1, wherein said protein does not contain Src homology 2 or arc homology 3 domains.
3. The protein of claim 1, wherein said protein comprises a serine and threonine rich moiety.
4. The protein of claim 1, wherein said protein comprises a kinase catalytic domain.
5. The protein of claim 1, wherein said protein is capable of activating MEK or MAPK protein.
6. The protein of claim 1, wherein said protein is encoded by a nucleic acid sequence, that encodes a protein capable of phosphorylating mammalian MEK independent of Raf protein.
7. The protein of claim 1, wherein said protein is used to produce antibodies capable of binding to MEKK protein.
8. The protein of claim 7, wherein said antibody is monoclonal.
9. A substantially pure protein encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1.
10. A substantially pure MEKK protein, capable of phosphorylating mammalian MEK protein, wherein said protein comprises a serine and threonine rich moiety and a kinase catalytic domain

and does not contain SH2 or SH3 domains.

11. The protein of claim 4, wherein said protein is capable of activating MEK or MAPK protein.
12. The protein of claim 4, wherein said protein comprises at least 672 amino acids.
13. The protein of claim 4, wherein said protein functions in the manner as the protein comprising the amino acid sequence shown in FIG. 1 (SEQ ID NO:1).
14. A substantially pure protein of claim 4 capable of phosphorylating MEK protein after about 40 minutes in a reaction mixture containing MgCl_2 and ATP, said mixture maintained at a pH of between about pH 6.0 and pH 8.0, said protein having an amino acid sequence distinct from Raf protein.
15. A substantially pure MEKK protein of claim 4, capable of regulating the activity of MAPK protein independent of Raf protein, said protein having an amino acid sequence distinct from said Raf protein.
16. A substantially pure MEKK protein of claim 1, capable of regulating signals initiated from a growth factor receptor on the surface of a cell by regulating the activity of MAPK protein, said ability to regulate being divergent from Raf protein signal regulation.
17. The protein of claim 16, wherein said growth factor receptor is coupled to heterotrimeric guanine nucleotide binding proteins.
18. The protein of claim 16, wherein said growth factor receptor is selected from the group consisting of thrombin receptors and muscarinic receptors.
19. A substantially pure MEKK protein of claim 4, which represents a divergence in mammalian cells from a yeast pheromone-responsive protein kinase system, said protein being capable of regulating MAPK protein independent of Raf protein.

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L2: Entry 1 of 1

File: USPT

Dec 29, 1998

US-PAT-NO: 5854043

DOCUMENT-IDENTIFIER: US 5854043 A

TITLE: MEKK-related signal transduction kinases

DATE-ISSUED: December 29, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Johnson; Gary L.	Boulder	CO		

US-CL-CURRENT: 435/194; 435/69.1, 530/350

CLAIMS:

What is claimed:

1. An isolated MEKK protein comprising the amino acid sequence of SEQ ID NO:4.
2. An isolated MEKK regulatory domain comprising amino acids 1-162 of SEQ ID NO:4.
3. An isolated MEKK catalytic domain comprising amino acids 351-619 of SEQ ID NO:4.
4. An isolated MEKK kinase domain comprising amino acids 386-480 of SEQ ID NO:4.
5. An isolated MEKK protein comprising the amino acid sequence of SEQ ID NO:6.
6. An isolated MEKK regulatory domain comprising amino acids 1-174 of SEQ ID NO:6.
7. An isolated MEKK catalytic domain comprising amino acids 357-626 of SEQ ID NO:6.
8. An isolated MFKK kinase domain comprising amino acids 392-486 of SEQ ID NO:6.
9. An isolated MEKK protein comprising the amino acid sequence of SEQ ID NO:8.
10. An isolated MEKK protein comprising the amino acid sequence of SEQ ID NO:10.
11. An isolated MEKK kinase domain comprising amino acids 656-742 of SEQ ID NO:8.

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L1: Entry 1 of 1

File: USPT

Dec 25, 2001

US-PAT-NO: 6333170

DOCUMENT-IDENTIFIER: US 6333170 B1

TITLE: Method and product for regulating cell responsiveness to external signals

DATE-ISSUED: December 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Johnson; Gary L.	Boulder	CO		

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 536/23.1, 536/24.31

CLAIMS:

What is claimed is:

1. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1.
2. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:3.
3. An isolated nucleic acid molecule which encodes a mitogen ERK kinase kinase (MEKK) protein comprising the amino acid sequence of SEQ ID NO:2.
4. An isolated nucleic acid molecule which encodes a mitogen ERK kinase kinase (MEKK) protein comprising the amino acid sequence of SEQ ID NO:4.
5. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:5.
6. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:7.
7. An isolated nucleic acid molecule which encodes a mitogen ERK kinase kinase (MEKK) protein comprising the amino acid sequence of SEQ ID NO:6.
8. An isolated nucleic acid molecule which encodes a mitogen ERK kinase kinase (MEKK) protein comprising the amino acid sequence of SEQ ID NO:8.
9. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:9.
10. An isolated nucleic acid molecule which encodes a mitogen ERK kinase kinase (MEKK) protein comprising the amino acid sequence of SEQ ID NO: 10.

11. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 11.
12. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 13.
13. An isolated nucleic acid molecule which encodes a mitogen ERK kinase kinase (MEKK) protein comprising the amino acid sequence of SEQ ID NO:12.
14. An isolated nucleic acid molecule which encodes a mitogen ERK kinase kinase (MEKK) protein comprising the amino acid sequence of SEQ ID NO: 14.
15. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a mitogen ERK kinase kinase (MEKK) catalytic domain selected from the group consisting of amino acids 409-672 of SEQ ID NO:2, 1329-1593 of SEQ ID NO: 4, 361-619 of SEQ ID NO:6, 361-619 of SEQ ID NO:8, 366-626 of SEQ ID NO:10, 631-890 of SEQ ID NO:12 and 1338-1597 of SEQ ID NO:14.
16. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a mitogen ERK kinase kinase (MEKK) NH.sub.2 -terminal regulatory domain selected from the group consisting of amino acids 1-408 of SEQ ID NO:2, 1-1328 of SEQ ID NO:4, 1-360 of SEQ ID NO:6, 1-360 of SEQ ID NO:8, 1-365 of SEQ ID NO: 10, 1-630 of SEQ ID NO:12 and 1-1337 of SEQ ID NO:14.

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L4: Entry 1 of 1

File: USPT

May 19, 1998

US-PAT-NO: 5753446

DOCUMENT-IDENTIFIER: US 5753446 A

**** See image for Certificate of Correction ****

TITLE: Mitogen ERK kinase kinase (MEKK) assay

DATE-ISSUED: May 19, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Johnson; Gary L.	Boulder	CO		

US-CL-CURRENT: 435/7.1; 435/252.3, 435/320.1, 435/325, 435/69.1, 530/300, 530/350, 536/23.1, 536/23.5

CLAIMS:

What is claimed:

1. An assay for identifying compounds which regulate signal transduction by a mitogen ERK kinase kinase (MEKK), comprising:

(a) providing a reaction mixture comprising a mammalian MEKK polypeptide;

(b) contacting the reaction mixture with a test compound; and

(c) determining the effect of the test compound on an indicator of signal transduction by the mammalian MEKK polypeptide in the reaction mixture to thereby identify a compound which regulates signal transduction by an MEKK.

2. The assay of claim 1, wherein the mammalian MEKK polypeptide comprises an amino acid sequence having at least 75% identity with a kinase catalytic domain of an MEKK polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12.

3. The assay of claim 1, wherein the mammalian MEKK polypeptide comprises an amino acid sequence having at least 85% identity with a kinase catalytic domain of an MEKK polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12.

4. The assay of claim 1, wherein the mammalian MEKK polypeptide comprises an amino acid sequence having at least 75% identity with a regulatory domain of an MEKK polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ

ID NO:10, and SEQ ID NO:12.

5. The assay of claim 1, wherein the mammalian MEKK polypeptide comprises an amino acid sequence having at least 85% identity with a regulatory domain of an MEKK polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, and SEQ ID NO:12.

6. The assay of claim 1, wherein the mammalian MEKK polypeptide is encoded by a nucleic acid molecule which hybridizes under highly stringent conditions with a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11.

7. An assay for identifying compounds having potential to regulate signal transduction by a mitogen ERK kinase kinase (MEKK), comprising:

(a) providing a reaction mixture comprising a mammalian MEKK polypeptide, wherein the reaction mixture is a cell or a cell-free mixture;

(b) contacting the reaction mixture with a test compound; and

(c) determining the effect of the test compound on an indicator of signal transduction by the mammalian MEKK polypeptide in the reaction mixture, wherein the indicator is interaction of the mammalian MEKK polypeptide with an MEKK interactor molecule or activity of a signaling pathway, to thereby identify a compound having potential to regulate signal transduction by an MEKK.

8. The assay of claim 1, wherein:

the reaction mixture comprises:

(i) a mammalian MEKK polypeptide,

(ii) an MEKK interactor molecule which binds to the mammalian MEKK polypeptide, and

(iii) a test compound; and the effect of the test compound on an indicator of signal transduction by the mammalian MEKK polypeptide in the reaction mixture is determined by:

detecting interaction of the mammalian MEKK polypeptide with the MEKK interactor molecule, wherein a change in the level of interaction of the mammalian MEKK polypeptide and MEKK interactor molecule in the presence of the test compound, relative to the level of interaction in the absence of the test compound, indicates that the test compound has the potential to regulate signal transduction by an MEKK.

9. The assay of claim 8, wherein the reaction mixture is a cell-free mixture.

10. The assay of claim 8, wherein the reaction mixture is a recombinant cell.

11. The assay of claim 8, wherein the MEKK interactor molecule is a polypeptide which specifically binds to the mammalian MEKK polypeptide.

12. The assay of any of claims 8, 9, or 10, wherein the mammalian MEKK polypeptide is a recombinant polypeptide.
13. The assay of claim 8, wherein the mammalian MEKK polypeptide includes a polypeptide sequence of an MEKK selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12.
14. The assay of any of claims 8, 9 or 10, wherein the step of detecting the interaction of the mammalian MEKK polypeptide with the MEKK interactor molecule includes detecting an enzymatic activity of the mammalian MEKK polypeptide.
15. The assay of any of claims 8, 9 or 10 wherein the step of detecting the interaction of the MEKK interactor molecule with the mammalian MEKK polypeptide comprises detecting, in the reaction mixture, the formation of complexes ("MEKK complexes") including the MEKK interactor molecule and the mammalian MEKK polypeptide.
16. The assay of claim 15, wherein at least one of the mammalian MEKK polypeptide and the MEKK interactor molecule comprises a detectable label, and the level of MEKK complexes formed in the test mixture is quantitated by detecting the label in at least one of the MEKK interactor molecule, the mammalian MEKK polypeptide, and the MEKK complexes.
17. The assay of any of claims 8, 9 or 10, wherein the step of detecting the interaction of the MEKK interactor molecule with the mammalian MEKK polypeptide comprises an immunoassay.
18. The assay of claim 10, wherein the step of detecting the interaction of the MEKK interactor molecule with the mammalian MEKK polypeptide comprises detecting an intracellular signal produced in a signal transduction pathway involving the mammalian MEKK polypeptide.
19. The assay of claim 18, wherein the recombinant cell includes a reporter gene sensitive to MEKK signal transduction.
20. The assay of claim 10, wherein the ability of the test compound to regulate apoptosis of a cell is measured.
21. The assay of claim 9, wherein the mammalian MEKK polypeptide is provided as a purified protein.
22. The assay of claim 9, wherein the mammalian MEKK polypeptide is provided as a cell lysate.
23. The assay of claim 8, wherein the MEKK interactor molecule comprises Ras or a portion thereof.
24. The assay of claim 8, wherein the MEKKF interactor molecule is selected from the group comprising MEK1, MEK2, MKK1, MKK2, MKK3, MKK4, JNK1, JNK2, SEK1, SEK2.
25. The assay of claim 10, wherein the recombinant cell includes a heterologous nucleic acid encoding the mammalian MEKK polypeptide.

26. The assay of claim 10, wherein the recombinant cell includes a reporter gene construct comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by interaction of the mammalian MEKK polypeptide and the MEKK interactor molecule.
27. The assay of claim 8, wherein the test compound is selected from the group consisting of: protein based, carbohydrate based, lipid based, nucleic acid based, natural organic based, synthetically derived organic based, and antibody based compounds.
28. The assay of claim 8, further comprising the step of preparing a therapeutic composition of a test compound identified in said assay.
29. The assay of claim 8, wherein the test compound is an inhibitor of the interaction between the MEKK interactor molecule and the mammalian MEKK polypeptide.
30. The assay of claim 8, wherein the test compound is a potentiator of the interaction between the MEKK interactor molecule and the mammalian MEKK polypeptide.